**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| In re application of: | Confirmation No. |
| Anthony Boey et al. | Examiner: Kishore |
| Application No.: 09/744,103 | Technology Center/Art Unit: |
| Filed: December 10, 2001 | ***Declaration of Ian Maclachlan, Ph.D. Under 37 C.F.R. § 1.132*** |
| For: LIPOSOMAL ENCAPSULATED NUCLEIC ACID-COMPLEXES | |
| Customer No.: 20350 | |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Ian MacLachlan, Ph.D., state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I hold a Ph.D. (1994) from the University of Alberta, and a Bachelor of Science degree (1988) from the University of Alberta. I am presently the Chief Scientific Officer for Protiva Biotherapeutics, Inc. (Burnaby, Canada).

3. My field of expertise is nucleic acid delivery and molecular gene therapy. I have authored over twenty-five publications in the field of nucleic acid delivery technology, molecular gene therapy and molecular genetics, and I am a member of the American Society of Gene Therapy and the Oligonucleotide Therapeutics Society.

4. Attached hereto as Exhibit A is a true copy of my *curriculum vitae* and a list of publications of which I am an author or co-author.

5. I have read and am familiar with the contents of the above-referenced patent application. In addition, I have read the Office Action, mailed March 30, 2005, received from the United States Patent & Trademark Office in the above-referenced patent application. It is my understanding that the Examiner is concerned that claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over U.S. Patent No. 5,908,777 ("Lee *et al.*"); that claims 1-6, 12-13, 15-17, 21-22, 28, 32-37, 39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over U.S. Patent No. 5,891,468 ("Martin"); that claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 are anticipated under 35 U.S.C. § 102(a) over Lee *et al.*, *J. Biol. Chem.* 271(14):8481-8487 (1996) ("Lee *et al.* 2"); that claims 11-14, 26-28, 30-31, 42, 52-53, 56, 578, and 62-63 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* and Lee *et al.* 2; that claims 17-22, 28-29, 45-48, 53-54, 60, and 63-64 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin further in view of U.S. Patent No. 5,885,613 ("Holland"); that claims 8-10, 23-25, 39-40, 50-51, and 61 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin further in view of U.S. Patent No. 6,420,176 ("Lisziewicz"); and that claims 65-66 are obvious under 35 U.S.C. § 103(a) over Lee *et al.* or Lee *et al.* 2 or Martin in combination with WO 98/20857 ("Papahadjopoulos"). For the reasons set forth herein, the Examiner's concerns are overcome.

6. This declaration is provided to clarify the distinguishing elements of the presently claimed invention and that the cited references, *i.e.*, Lee *et al.*, Martin, and Lee *et al.* 2, neither teach nor suggest all of the elements of the presently claimed liposomes and, accordingly, do not anticipate the presently claimed invention. This declaration is further provided to demonstrate that the secondary references, *i.e.*, Holland, Lisziewicz, and Papahadjopoulos do not remedy the defects in the cited references and, accordingly, do not render the presently claimed invention obvious.

7. The present invention is directed to liposomes comprising a lipid and a condensing agent-nucleic acid complex ***encapsulated*** within the liposome.

8. It is my understanding that Lee *et al.*, Martin, and Lee *et al.* 2 are each cited by the Examiner as allegedly disclosing condensed nucleic acid preparations encapsulated within a liposome and thus, as allegedly anticipating the presently claimed invention. I have

reviewed Lee *et al.*, Martin, and Lee *et al.* 2, and, as discussed in detail below, ***none*** of Lee *et al.*, Martin, or Lee *et al.* 2, teaches or even suggests a liposome comprising a condensing agent-nucleic acid complex ***encapsulated*** within the liposome. It is also my understanding that Holland, Lisziewicz, and Papahadjopoulos are cited by the Examiner as secondary references that in combination with Lee *et al.*, Martin, or Lee *et al.* 2, allegedly render the presently claimed invention obvious. I have also reviewed Holland, Lisziewicz, and Papahadjopoulos and, as discussed in detail below, none of the secondary references supply the teachings absent in Lee *et al.*, Martin, or Lee *et al.* 2. Accordingly, the combination of the secondary references with Lee *et al.*, Martin, or Lee *et al.* 2, does not render the presently claimed invention obvious.

9. Lee *et al.* discloses nucleic acid-lipid complexes comprising anionic liposomes (*see*, col. 8, lines 7-9) and nucleic acid-polylysine complexes (*see*, col. 8, lines23-24). Only after the liposomes are fully formed are they mixed with nucleic acid-polylysine complexes (*see, e.g.*, col. 8, lines 27-29) in deionized water. Lee *et al.* characterizes the interaction between the fully formed liposomes and nucleic acid-polylysine complexes in the liposomes as encapsulation of the nucleic acid-polylysine complex. However, given that DNA does not readily cross lipid membranes, one of skill in the art would appreciate that mixing of a nucleic acid-polylysine complex with preformed liposomes in an aqueous solution does ***not*** result in entrapment of DNA within the internal space of the liposomes, but would, instead, result in formation of nucleic acid-lipid ***complexes***. Without a step that destabilizes the liposome membrane, the nucleic acid would ***not*** be able to enter the liposome and be encapsulated. Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid ***complexes*** of Lee *et al.* do not comprise a nucleic acid fully encapsulated in a liposome.

10. Martin also discloses nucleic acid lipid complexes. As explicitly set forth in Example 9, preformed liposomes are ***complexed*** to plasmid-histone complexes (*see*, Example 9 at col. 29, lines 41-42). Thus, Martin does not describe nucleic acid-histone complexes fully encapsulated in a liposome. Martin also describes the use of dehydration-rehydration-extrusion methods to allegedly encapsulate plasmid-histone complexes (*see*, Example 11 at col. 31, lines 11-26). However, as demonstrated in paragraph 16, below, such methods do not produce liposomes encapsulating nucleic acids. Thus, in contrast to the liposomes of the present

invention, the nucleic acid-lipid complexes of Martin do not comprise a nucleic acid fully encapsulated in a liposome.

11. Lee *et al.* 2 discloses nucleic acid-lipid complexes that are the same or similar to those set forth in Lee *et al.* As set forth in Lee *et al.* 2, preformed anionic liposomes are mixed with nucleic acid-polylysine complexes in deionized water (*see, e.g.*, page 8482, col. 2, lines 13-15). As discussed above in paragraph 9, one of skill in the art would appreciate that mixing preformed liposomes with nucleic acid-polylysine complexes in an aqueous solution would result in formation of lipoplexes, *i.e.*, complexes between the liposomes and nucleic acid-condensing agent, and not liposomes fully encapsulating a nucleic acid. Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid complexes of Lee *et al.* 2 also do not comprise a nucleic acid fully encapsulated in a liposome.

12. None of the secondary references, *i.e.*, Holland, Lisziewicz, or Papahadjopoulos remedy the defects in Lee *et al.*, Martin, or Lee *et al.* 2. Holland is cited by the Examiner as disclosing the use of PEG-ceramide as a liposome bilayer stabilizing component; Lisziewicz is cited by the Examiner as disclosing the use of PEI as a nucleic acid condensing agent; and Papahadjopoulos is cited by the Examiner as disclosing preparation of liposomes by reverse phase evaporation or detergent dialysis. Each of these allegations is addressed in detail below.

13. Holland discloses the use of PEG-ceramide in a nucleic acid lipid ***complex***. More particularly, Holland *et al.* states:

> Cationic lipids have been used in the transfection of cells in vitro and in vivo. . . .The efficiency of this transfection has often been less than desired, for various reasons. ***One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.***
>
> See, column 12, lines 28-39 of Holland et al. (emphasis added).

Clearly, the teachings of Holland are directed to forming nucleic acid-cationic liposome ***complexes***, which are structurally and functionally different from the presently claimed liposomes, wherein the nucleic acid-condensing agent complex is encapsulated in the liposome is resistant in aqueous solution to degradation with a nuclease. Moreover, as discussed in detail in

paragraph 16 below, the methods set forth in Holland cannot not be used to encapsulate a nucleic acid in a liposome.

14. Lisziewicz discloses nucleic acid-condensing agent complexes and compares the efficiency and toxicity of PEI and PEI-mannose as a condensing agent (*see, e.g.*, col. 15, lines 45-50). Lisziewicz explicitly states that relative to PEI mannose, PEI (1) is more toxic; (2) requires more DNA to neutralize; and (3) is less efficient for transfection. Thus, based on the disclosure of Lisziewicz, one of skill in the art would not use PEI as a nucleic acid condensing agent. Moreover, in contrast to the presently claimed invention, Lisziewicz does not teach or suggest liposomes comprising a nucleic acid-condensing agent complex encapsulated in the liposome.

15. Papahadjopoulos discloses nucleic acid-lipid complexes formed by mixing preformed liposomes with nucleic acids (*see, e.g.*, page 35, lines 1-21). As explained in detail above, mixing preformed liposomes with nucleic acids will lead to formation of lipoplexes, *i.e.*, complexes between the nucleic acids and liposomes, and will ***not*** lead to encapsulation of the nucleic acid in the liposomes. In fact, the disclosure of Papahadjopoulos explicitly states that the methods described therein are used for forming complexes between preformed liposomes and nucleic acids (*see, e.g.*, page 8, lines 28-29 and page 13, lines 4-17). Papahadjopoulos does not disclose or suggest encapsulating nucleic acids in liposomes using detergent dialysis or reverse phase evaporation.

16. To further demonstrate that the dehydration-rehydration-extrusion methods set forth in Martin and Holland do ***not*** produce the presently claimed liposomal formulations, a series of experiments using such methods were conducted under my supervision. A lipid solution containing a total of 2.22 μmoles lipid and comprising DOPE:DODAC:PEG-ceramide C14 (82.5:7.5:10 molar percent), was prepared by dissolving these lipids in chloroform. Nitrogen gas was used to drive off chloroform to form a lipid film. The lipid film was then hydrated with 2 ml phosphate buffered saline (pH 7.4) containing 50 or 100 μg of nucleic acid (*i.e.*, plasmid DNA) to generate liposomal samples with drug (*i.e.*, nucleic acid):lipid ratios of 22.5 and 45 μg input DNA/μmol. The resulting suspension was subjected to 5 rounds of freezing in liquid nitrogen and thawing in a 37°C water bath, to increase homogeneity of the resulting multilamellar vesicles. The multilamellar vesicles were all greater than 10,000 nm in diameter. To produce liposomes of appropriate size, the samples were then extruded 10 times through 2 stacked 100

nm polycarbonate filters using a 10-mL Extruder (Northern Lipids Inc.) and nitrogen gas at 400-600 psi. Nucleic acid encapsulation was determined using membrane-impermeable Picogreen which fluoresces in the presence of plasmid DNA. The proportion of nucleic acid encapsulated in the liposomes was determined by measuring the fluorescence intensity of the Picogreen before and after the addition of the detergent Triton X-100.

As set forth in Exhibit B, plasmid encapsulation and recovery were both extremely inefficient at both of the input nucleic acid amounts examined. Specifically, prior to extrusion, only 12% or 15% of the input nucleic acid was inaccessible to Picogreen due to its association with or incorporation into >10,000 nm multilamellar vesicles (*see*, Exhibit B). In addition, only 1.4% or 2% of the input nucleic acid was actually recovered after the extrusion step necessary to form actual liposomes (*see*, Exhibit B). Furthermore, only 0.055% or 0.14% of the input nucleic acid was recovered and encapsulated post extrusion. Particle sizes for all of these extruded samples were all considerably larger than 100 nm. These results unequivocally demonstrate that dehydration-rehydration-extrusion methods do ***not*** produce liposomes that encapsulate plasmid DNA.

17. In view of the foregoing, it is my opinion that Martin and Holland do not teach (or even suggest) the presently claimed liposomes because Martin and Holland do not teach (or even suggest) (1) liposomes wherein the nucleic acid is encapsulated in the liposome and is resistant in aqueous solution to degradation with a nuclease, or (2) methods for making such liposomes. Moreover, it has been unequivocally demonstrated that the dehydration-rehydration-extrusion methods described in Martin and Holland do not lead to the presently claimed liposomes wherein the nucleic acid-condensing agent complex is encapsulated in the liposome.

18. In view of the foregoing, it is my opinion that ***none*** of the cited references, *i.e.*, the Lee *et al.* patent, the Martin patent nor the Lee *et al.* publication teach (or even suggest) the presently claimed liposomal formulations because ***none*** of Lee *et al.*, Martin, nor Lee *et al.* 2 teach (or even suggest) liposomes, wherein the nucleic acid is encapsulated in the liposome and is thus resistant in aqueous solution to degradation with a nuclease. In contrast to the teachings of Lee *et al.*, Martin, and Lee *et al.* 2, the present invention provides novel liposomes which are produced by forming a complex between a nucleic acid (*e.g.*, oligonucleotides, plasmid DNA, *etc.*) and a condensing agent and the resulting complex is ***encapsulated***, within a liposome. This is in stark

contrast to the lipoplexes that would be formed based on the using the methodology set forth in each of the cited references.

It is also my opinion that none of the secondary references, *i.e.*, Holland, Lisziewicz, or Papahadjopoulos remedy the defects of Lee *et al.*, Martin, and Lee *et al.* 2 because none of the secondary references teach (or even suggest) liposomes wherein a nucleic acid is encapsulated in the liposome and is therefore resistant in aqueous solution to degradation with a nuclease.

19. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: September 30, 2005

Ian MacLachlan, Ph.D.

# CURRICULUM VITAE
# IAN MACLACHLAN, PH.D.

## BIOGRAPHIC DATA


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## EDUCATION

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|---|---|
| May 1988 - Jun 1994 | **Ph.D. (Biochemistry)**<br>University of Alberta, Edmonton, Canada,<br>& Department of Molecular Genetics, University of Vienna, Austria. |
| Sep 1985 - May 1988 | **B.Sc. (Biochemistry)**<br>University of Alberta, Edmonton, Canada. |
| Sep 1982 - May 1984 | **Biological Sciences**<br>University of Calgary, Calgary, Canada. |

## EXPERIENCE

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|---|---|---|
| Sep 2000 - Present | **Chief Scientific Officer**<br>Protiva Biotherapeutics, Inc.,<br>Burnaby, BC, Canada. | Development of Non-Viral Nucleic Acid Delivery Systems for Cancer, Inflammatory and Infectious Disease. |
| Jul 1996 - Aug 2000 | **Team Leader / Research Scientist**<br>Inex Pharmaceuticals Corporation<br>Burnaby, BC, Canada. | Non-Viral Cancer Gene Therapy.<br>Suicide Gene Therapy, Pharmacology, Vector Development, Tumor Modeling, Inducible Gene Expression. |
| Jul 1994 - Jun 1996 | **Research Fellow**<br>Howard Hughes Medical Institute<br>Department of Internal Medicine<br>University of Michigan, USA.<br>Supervisor: Dr. G.J. Nabel | TNF Mediated Activation of NF-κB and the HIV LTR<br>Adenoviral Gene Therapy for Restenosis.<br>The Role of NF-κB in Vertebrate Development. |
| May 1988 - Jun 1994 | **Graduate Student**<br>Lipid and Lipoprotein Research Group<br>University of Alberta, AB, Canada.<br>& Dept. of Molecular Genetics<br>University of Vienna, Austria.<br>Supervisor: Dr. Wolfgang Schneider | Molecular Genetics of the Lipoprotein Receptor Family.<br>Characterization of the Receptor Mediated Uptake of Riboflavin Binding Protein Including Cloning and Characterization of the *rd* Mutant. |
| Jan 1988 - Apr 1988 | **Undergraduate Research**<br>University of Alberta, AB, Canada.<br>Supervisor: Dr. Wayne Anderson | Computerized Sequence Analysis of Lipoproteins,<br>Crystallography of Membrane Proteins. |

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| Sep 1987 - Dec 1987 | **Undergraduate Research**<br>University of Alberta, AB, Canada.<br>Supervisor: Dr. Wolfgang Schneider | Purification and Characterization of Apolipoprotein VLDL-II, an Inhibitor of Lipoprotein Lipase. |
| Summer 1987 | **Undergraduate Research**<br>Bamfield Marine Station, Canada.<br>Supervisor: Dr. Ron Ydenberg | Behavioral Analysis of the Polychaete, *Eudystilia vancouveri.* |
| May 1983 - Dec 1986 | **Programmer**<br>Canadian Hunter Exploration Ltd.<br>Calgary, Alberta, AB, Canada. | Programming of Oil and Gas Reservoir Simulations and Data Analysis Tools Used to Guide the Exploration Efforts of an Oil and Gas Company. |

## TRAINING

| | | |
|---|---|---|
| June 2004 | American Society of Gene Therapy/ USFDA | Long Term Follow-up of Participants in Human Gene Transfer Research |
| March 2003 | American Society of Gene Therapy / USFDA | Non-Clinical Toxicology in Support of Licensure of Gene Therapies |
| Sept 2002 | Protiva Biotherapeutics | WHMIS and Chemical Safety Retraining |
| Sept 2002 | TLM Consulting | Basic GMP Training |
| June 2002 | American Society of Gene Therapy / USFDA | Clinical Gene Transfer Comprehensive Review Course |
| Apr 2002 | TLM Consulting | Introduction to Gene Therapy Clinical Trials and GLP/GMP |
| Jul 2001 | Protiva Biotherapeutics | Cytotoxic Drug Training |
| May 2001 | American Society of Gene Therapy / USFDA | Clinical Gene Transfer Training Course |
| Jun – Sep 1998 | Leadership Edge Consulting | Lab-to-Leader Training Program<br>Project Management, Coaching, Team Management |
| Oct 1997 | Pape Management Consulting | Project Management Training II |
| May 1997 | University of British Columbia | Radionuclide Safety and Methodology |
| Feb 1997 | Pape Management Consulting | Project Management Training I |

## AWARDS AND DISTINCTIONS

| | |
|---|---|
| 1995 - 1998 | Medical Research Council of Canada Fellowship |
| 1993 | Mary Louise Imrie Graduate Award, Faculty of Graduate Studies and Research, University of Alberta |
| 1992 - 1994 | Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (Austrian Ministry of Science Scholarship) |
| 1989 - 1993 | Heart and Stroke Foundation of Canada Research Trainee |
| 1982 | Rutherford Scholarship |

## AFFILIATIONS AND MEMBERSHIPS

| | |
|---|---|
| 1999 - 2002 | Science Council of British Columbia - Health Technology Committee |
| 1998 - Present | American Society of Gene Therapy, Member |
| 2004 - Present | American Society of Gene Therapy - Non-viral Vectors Committee |

## PUBLICATIONS

Judge, A.D., Sood, V., Shaw, J.R., Fang, D., McClintock, K. and MacLachlan, I., *Synthetic siRNA Stimulate the Mammalian Innate Immune Response in a Sequence Dependent Manner*, In Press: Nature Biotech, 2005.

Heyes, J., Palmer, L.R., Bremner, K. and MacLachlan, I., *Cationic Lipid Saturation Influences Intracellular Delivery of Encapsulated Nucleic Acids*, In Press: Journal of Controlled Release, 2005.

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Jeffs, L.B., Palmer, L.R., Ambegia, E.G., Giesbrecht, C., Ewanick, S. and MacLachlan, I., *A Scalable, Extrusion Free Method for Efficient Liposomal Encapsulation of Plasmid DNA*, In Press: Pharmaceutical Research, 2005.

MacLachlan, I. and Cullis, P.R., Diffusible-PEG-Lipid Stabilized Plasmid Lipid Particles, In Press: In: Non-viral Vectors for Gene Therapy, Huang, L., Hung, M.C. and Wagner, E., Eds. Academic Press, 2005.

Finn, J., MacLachlan, I., Cullis, P.R., *Factors Limiting Autogene-based Cytoplasmic Expression Systems*, In Press: FASEB Journal, 2005.

Finn, J., Lee, A., MacLachlan, I., Cullis, P.R., *An Enhanced Autogene-based Dual Promoter Cytoplasmic Expression System Yields Increased Gene Expression*, Gene Ther. 2004 Feb;11(3):276-83.

Palmer, L.R., Chen, T., Lam, A.M.I., Fenske, D.B., Wong, K.F., MacLachlan, I., Cullis, P.R., *Transfection Properties of Stabilized Plasmid-Lipid Particles Containing Cationic PEG Lipids*, Biochim Biophys Acta.1611:204-16, 2003.

Cullis, P.R., MacLachlan, I., Fenske, D.B., *Lipid Based Systems for Systemic Gene Therapy*, Journal of Liposome Research, In Press.

Fenske, D.B., MacLachlan, I., Cullis, P.R., *Stabilized Plasmid-Lipid Particles: a Systemic Gene Therapy Vector*, Methods in Enzymology, 346: 36-71, Academic Press, San Diego, 2002.

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Fenske, D.B., MacLachlan, I., Cullis, P.R., *Long-circulating Vectors for the Systemic Delivery of Genes*, Current Opinion in Molecular Therapeutics, 3 (2):153-158, 2001.

Pampinella, F., Pozzobon, M., Zanetti, E., Gamba, P.G., MacLachlan, I., Cantini, M., Vitiello, L., *Gene Transfer In Skeletal Muscle by Systemic Injection of DODAC Lipopolyplexes*, Neurological Science, 21:S971-973, 2000.

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Elkin, R.G., MacLachlan, I., Hermann, M., Schneider, W.J., *Characterization of the Japanese Quail Oocyte Receptor for Very Low Density Lipoprotein and Vitellogenin*, J. Nutrition, 125: 1258 - 1266, 1995.

MacLachlan, I., Nimpf, J., Schneider, W. J., *Japanese Quail Apo-VLDL-II: cDNA Sequence and Comparison to Chicken Apo-VLDL-II, a Specific Inhibitor of Lipoprotein Lipase.* Atherosclerosis: 109: 62,1994.

MacLachlan, I., Schneider, W.J., *Avian Riboflavin Binding Protein Binds to Lipoprotein Receptors in Association With Vitellogenin.* J. Biol. Chem., 269: 24127-24132, 1994.

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**SELECTED ABSTRACTS**

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MacLachlan, I., Ambegia, E., McClintock,.K, Jeffs, L., Palmer, L., Meitz, A., &. Cullis, P.R, *Disease Site Targeting and Tumor Specific Gene Expression of Stable Plasmid-Lipid Particles for Systemic Gene Delivery,* Eleventh International Conference on Cancer Gene Therapy, July 11-12, 2002.

Finn, J.D., Lee, A., MacLachlan, I., Cullis, P.R. *The Development and Characterization of a Cytoplasmic Expression System Based on the T7 Phage RNA Polymerase Protein.* American Society of Gene Therapy, 5th Annual Meeting, June 5-9, 2002.

Sandhu, A., Verheul, R., de Jong, S., MacLachlan, I., Cullis, P. *Enhancing the Intracellular Delivery Characteristics of Stable Plasmid-Lipid Particles.* American Society of Gene Therapy, 5th Annual Meeting, June 5-9, 2002.

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Wong, T., Wong, K., Cullis, P., Fenske, D., MacLachlan, I., Sandu, A., Lo, E. *Optimizing the Transfection Potency of Stable Plasmid-Lipid Particles Based on the Endosomal Release Parameter.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Cullis, P., Finn, J., MacLachlan, I. *The Development and Comparison of Three Cytoplasmic Expression Systems Based on the T7, T3 and SP6 Phage RNA Polymerase Proteins.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Ansell, S., Currie, K., Ambegia, E., Cullis, P., Carr, K., MacLachlan, I., Murray, M. *Stabilized Plasmid Lipid Particles Containing Diacylglycerol Anchored PEG Lipids:In vitro and In Vivo Characterization.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Ambegia, E., Cullis, P., Fenske, D., Palmer, L., MacLachlan, Ian., Murray, M. *Programmable Pharmacokinetics, Disease Site Targeting and Tissue Specific Gene Expression of Stable Plasmid-Lipid Particles for Systemic Gene Delivery and Expression.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Finn, J., MacLachlan, I., Cullis, P. *The development and comparison of three cytoplasmic expression systems based on the T7, T3 and SP6 phage RNA polymerase proteins.* Gene Therapy 2001: A Gene Odyssey, January 6-11, 2001.

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Ahkong, L., Airess, R., Harasym, T. Hope, M., Klimuk, S., Leng, E., MacLachlan, I., Semple, S.C., Tam, P. and Cullis, P.R., *Pre-clinical Studies with Liposomal Mitoxantrone: Formulation, Pharmacokinetics, Toxicity and Efficacy,* 7th Liposome Research Days, April 12-15, 2000.

MacLachlan, I., Tam, P., Lee, D., Thompson, J., Geisbrecht, C., Lee, A., Thomson, V. and Cullis, P.R., *A Gene Specific Increase In the Survival of Tumor Bearing Mice Following Systemic Non-viral Gene Therapy,* 7th Liposome Research Days, April 12-15, 2000.

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# Exhibit B

| Sample | % Input Nucleic Acid Inaccessible to Picogreen Prior to Extrusion | % Input Nucleic Acid Recovered Post-Extrusion | % Input Nucleic Acid Recovered and Encapsulated Post-Extrusion |
|---|---|---|---|
| Empty vesicles | n/a | n/a | n/a |
| Plasmid DNA 22.5 µg/µmol | 32 ± 6 | 1.4 ± 0.5 | 0.055 ± .475 |
| Plasmid DNA 45 µg/µmol | 12 ± 5 | 2.0 | 0.14 |